DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS

i 141-5 (OPPTS 850. 3040)

1. CHEMICAL: Clothianidin PC Code No.: 044309

2. TEST MATERIAL: Clothianidin FS 600B G Purity: 595 g/L

3. CITATION:

Author: Liepold, K.

Title: Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Champagne (France).

Study Completion Date: January 5, 2010

Laboratory: Eurofins-GAB GmbH, Niefern, Oschelbronn, Germany

Sponsor: Bayer CropScience AG, Ecotoxicology, Monheim, Germany

Laboratory Report ID: S09-01403

DP Barcode: 374484

MRID No.: 47972302

4. REVIEWED BY: Moncie Wright, Staff Scientist, Cambridge Environmental, Inc.

Signature: Moncie V Wright **Date:** 08/01/11

APPROVED BY: Teri S. Myers, Ph.D., Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date**: 08/01/11

5. APPROVED BY: Allen Vaughan, Biologist, ERB - V

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the long-term toxicity of a pesticide to honey bees following an actual-use field exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Queens in all colonies were of the same lineage and the bees in all colonies were young.
Definitive Study Duration:	79 days (11 day exposure period to first drilling that occurred 2-3 days earlier, then a 34-day exposure period after the 2nd drilling, followed by a 34-day post-exposure period).

8. CONCLUSIONS:

In a 79 day study (11 day exposure period to first drilling that occurred 2-3 days earlier, then a 34-day exposure period after the 2nd drilling, followed by a 34-day post-exposure period), the toxicity of dust from clothianidin-treated seed during drilling of treated maize seeds was examined in the honey bee, *Apis mellifera* L., under open field conditions at two test sites (the treatment plots were located at Bouy, near Chalons-en-Champagne), in the region of Champagne, France.

The treated site was planted with maize seeds dressed with the end-use product Clothianidin FS 600B G (AI: 595 g/L Clothianidin), and the other site was planted with untreated control seed. The treatment and the control plots were separated by *ca*. 2 km. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha. Six honeybee colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area (<1 m for the control) with the entrance facing the maize field.

The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 11 days before the second drilling and remained at the study location for 34 days after seeding. For the post exposure period the colonies were moved from the exposure plots to a monitoring location near Wissembourg, Alsace, France. Throughout the study, colonies were assessed for mortality, colony strength, and brood and food store area. Additionally, the occurrence and duration of guttation, flight activity and bee behavior, and bees collecting guttation liquid were also observed.

The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. Generally, guttation occurred at a similar rate over the 4 zones that were assessed, but did not occur at a similar rate between the control and treatment plots. In general, the occurrence of guttation was more pronounced in the treatment plot compared to the control plot. Dew and guttation did not occur together on all assessment days. Generally, there were more days with occurrences of guttation only as compared to days with both guttation and dew.

The period of guttation and bee activity overlapped. The behavior of the bees in front of the hives was normal for all observation periods in the control and treatment plots. No honeybees were observed consuming guttation liquid in the control or treatment plot for the entire duration of the study period.

For the remaining assessment days, mean daily mortality of both the control and treatment groups fluctuated. Mortality peaks usually occurred simultaneously in both the control and treated plots. The mean daily mortality during guttation (days 11 to 34 after drilling) was 11.4 and 9.5 dead bees/hive in the control and treated groups, respectively. The mean daily mortality (linen sheets + bee traps) for the entire exposure (34 days) was 12.7 and 11.1 bees/hive in the control and treated groups, respectively.

Colony strength fluctuated throughout the exposure period in both the control and treatment groups. The colony strength in the treatment group was comparable to that of the control group on most assessments. Those days where colony strength was possibly significantly reduced as compared to the control there was swarming that likely resulted in loss of the queen and numerous worker bees. On the last assessment day, the assessment was so near to the reported

dates of swarming that the colony likely did not have sufficient time to recover. Therefore, any reductions cannot be conclusively attributed to the drilling of the treated maize seeds.

The brood and food area data were very similar between the treatment and control groups, and both colonies demonstrated similar fluctuations in the mean abundance of brood on the combs. The cases where there might have been significant reductions in the treatment as compared to the control coincided with the swarming incident that was reported, and thus there was likely an effect on the brood and food area as well.

It was concluded that no adverse effects of the potential exposure of honeybee colonies to dust generated during drilling of treated maize seeds and to guttating maize on colony health and development was observed during exposure and in the 34 day post exposure monitoring phase. Further, no obvious treatment related differences were observed between control and treatment group mortality during exposure.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Guttation fluid, dead honeybees and pollen and nectar from combs were not analyzed because the study authors determined there was no damage to individual bees or bee colonies due to clothianidin-treated maize exposure.

This study is scientifically sound and *satisfies/does not satisfy* the EFED concerning the guideline requirements for a field toxicity test with honeybees (Subdivision L, i 141-5 or 850.3040).

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable / Supplemental / Unacceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: There were no guideline deviations.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of clothianidin to honeybees in a field test for the purpose of chemical reregistration.

Specifically, the test was conducted to determine the relevance of potentially occurring guttation in young maize plants in the Champagne region in France as a water source for honeybees, and to assess potential effects of Clothianidin residues from the seed treatment of the maize seeds in guttation liquid on bee colonies under field conditions. Additionally, assessments were performed on the potential effects of the maize drilling process during which the colonies might be exposed to Clothianidin-containing dust from the seed treatment.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: **Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)**	*Apis mellifera* L. (Hymenoptera, Apidae)
Colony description at beginning of test:	Each colony occupied hives consisting of two boxes (lower box=brood chamber=1; upper box=honeycomb box=2) that included 10

Guideline Criteria	Reported Information
	combs each. Queens in all colonies were of the same lineage and approximately the same age. A queen excluder was placed between the brood chamber and honeycomb box to retain the queen in the brood chamber. There was 1 queen per colony and between 5,382 and 15,197 bees per colony at study initiation.
Pre-test health:	Bees were reportedly free of *Nosema* and *Varroa* disease symptoms.
Supplier	The colonies were supplied by a beekeeper, Mr. Scheible-Marz, Eislingen, Germany
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Exposure Site Location and Establishment:	The test fields were located at Bouy, near Chalons-en-Champagne, in the region of Champagne, France. Both the control and treatment plots were divided into two parts, on which drilling took place on two different, subsequent dates. The treated site was planted with clothianidin-dressed maize seed and the other planted with untreated control seed. The treatment and the control plots were separated by *ca*. 2 km. The size of the field plots was 1.72 ha for the treated plot and 1.91 ha for the control.

Guideline Criteria	Reported Information
	The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha. Effective rates: Control: 102,500 seeds/ha Treatment: 103,800 seeds/ha
Site Preparation:	Pre-hive set-up maize drilling was performed on April 28-29, 2009.
Number of applications:	Two; drilling occurred on April 28-29, 2009 before the placement of the hives 2-3 days later, and on May 12, 2009 after hive set-up on the plots.
Number of Replicates/Treatment:	Six colonies per field plot, with 1 treated and 1 control field plot
Post-exposure Site Location:	Near Wissembourg, Alsace, France.
Lighting:	Natural; not further described.
Precipitation:	Precipitation measured during mortality assessments at the control plot ranged from 0.0 to 11 L/m^2 during the exposure period (data obtained from Figure 23). The maximum rainfall events occurred on May 14 and June 11, 2009 when 11 L/m^2 precipitation occurred.
Temperature:	Daily temperatures ranged from 0.6 to 29.8°C during the exposure period.
Relative humidity:	Mean relative humidity ranged from 25 to 100% during the exposure period.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported
Reference toxicant tested?	No
Duration of Exposure Period	34 days

Guideline Criteria	Reported Information
Duration of Post-exposure Period	34 days in the monitoring site
Test Substance(s):	Clothianidin FS 600B G Formulation Type: suspension Batch No.: PF90191228 AI: 595 g/L Clothianidin (analyzed)
Control Substance(s):	N/A- control seeds were not treated
Maize Seed:	Seed variety: TEXXUD
Application Rate:	0.506 mg ai per seed (analyzed)
Verification of Application Rate:	Not reported
Method of Seed Coating:	Not reported
Colony Introduction:	The colonies were placed at the edge of each field plot at a distance of 1-2 m from the sowing area (<1 m for the control) with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 11 days before the second drilling and remained at the study location for 34 days after seeding.

Guideline Criteria	Reported Information
Post-exposure:	The colonies were moved from the exposure plots to a monitoring location near Wissembourg, Alsace, France.
Assessment scheme:	The part of the field plots that was considered to be most likely to be attractive to honeybees seeking water was assessed regarding the occurrence of guttation and/or dew (assessment area). The in-field assessment area (zones 1-4) covered a width of 5 m to the left and to the right from the outer bee hives at each field, and in length encompassed 58 parallel rows of maize (45.6 m). At the treatment plot, the assessment area started in row 5 because the first 4 rows were not sown; all zones were shifted for 4 rows. Each assessment started with zone 0 and ended with zone 4.
Assessment zones:	Zone 0 = off-field assessment area; between row number 1; 2-4 m away from the field. Zone 1 = rows 1-7 in the control plot and rows 5-11 in the treated plot; assessments were performed along each row; observers made assessments while walking. Zone 2 = rows 8-13 for the control and rows 12-17 for the treated plot; assessments were made for rows in groups of 3 (each 3rd row was a passing row). Zone 3 = rows 14-28 for the control and 18-32 for the treated plot; assessments were made for rows in groups of 5 (each 5th row was a passing row). Zone 4 = rows 29-58 for the control and 33-63 for the treated plot; assessments were made for rows in groups of 5 (each 10th row was a passing row). Additionally, there were six 2 m^2 plots that

Guideline Criteria	Reported Information
	each covered 2 rows of maize seedlings.

D. Biological Assessments

Guideline Criteria	Reported Information
Maize guttation:	The proportion of maize plants displaying guttation and/or dew was monitored as the maize seedlings emerged and lasted for 24 days. This was determined by observers that walked through each passage row. The percentage was estimated at 10, 25, 50, 75, 90, and >90%. If less than 10% of the plants displayed guttation, the exact number of plants in an assessment row that showed guttation was counted. Guttation occurrence was checked in regular intervals from the early morning shortly after sunrise. One full observation period included the guttation assessments in the 4 established zones in the fields. Additionally, zone 0 was checked for the presence of guttation and/or dew on the off-field vegetation and to determine if the extent of guttation and/or dew on the off-field vegetation was more or less than that present on the plants in the maize field. If no guttation occurred at both field sites then the plants of neighboring fields or adjacent vegetation were checked for guttation
Bees collecting guttation droplets:	The bees were monitored as the maize seedlings emerged and lasted for 24 days. After the assessment of guttation and honeybee activity in the zones the number of honeybees per assessment plot sitting on the ground or on

Guideline Criteria	Reported Information
	plants, and the number taking up droplets was recorded during a 4 minute assessment period per plot. Any abnormal behavior was documented.
Flight activity:	On each assessment day (those days on which guttation was observed), the flight activity at the hive entrance of each hive was documented at the start and end of each observation period. Flight activity was assessed by counting the number of bees entering the hive over 1 minute and by counting the number leaving the hive over 1 minute.
Mortality:	Linen sheets were spread on the ground in front of the hives and dead bee traps were attached to the entrance of each hive to measure mortality during the exposure period. Mortality was assessed four days before drilling, on the day of seeding (after seeding was done), and daily thereafter until the termination of the exposure phase. The dead bee traps were emptied daily at the same time of day and the bees were transferred within 10 hours into a deep freezer (≤-18°C) for potential residues analysis.

Guideline Criteria	Reported Information
Colony condition:	The condition of the colonies was recorded once before the hives were placed on the field plots and afterwards in weekly intervals during the exposure phase.
Brood:	During the monitoring phase the brood assessments were performed 4 times in weekly intervals. The following parameters were assessed: - Colony strength (number of bees) - Presence of a healthy queen (presence of eggs) - Pollen storage area and area with nectar or honey - Area containing cells with eggs, larval, and capped cells The comb area covered with bees and cells with nectar, pollen, egg, larval, and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen, and nectar on the comb was calculated. The mean values were calculated for each hive and assessment date.
Collection of guttation fluid:	Guttation fluid was sampled on days when sufficient guttation for sampling was available early in the morning in the treated plot. The samples were collected in the morning within the first hour of the assessments on the field outside the guttation assessment areas and in a distance of at least 20 m from the hives. The fluid was collected with plastic Pasteur pipettes and was stored in Eppendorf caps. Samples were stored on blue ice and transferred within 14 hours to a deep freezer (≤-18°C). During the trial, sampling occurred on 20 days.

Guideline Criteria	Reported Information
Collection of pollen and nectar from combs:	Samples of pollen and nectar were collected from the bee hive combs during each brood assessment after drilling during the exposure phase. If possible, one sample that weighed 1 gram was taken per colony in the control and treated plots. Each sample was taken from 3 different sections per hive, and then all 3 samples were pooled. Pieces of comb were cut from the comb using a clean knife for each sample. A spoon was used to collect nectar. Samples were stored cooled and transferred within 10 hours to a deep freezer (≤-18°C). No further preparation was performed because the residues were not analyzed.

E. Residue Analysis

Guideline Criteria	Reported Information
Guttation fluid, dead bees, pollen and nectar from combs:	The study author concluded that Clothianidin-treated maize did not have negative effects on any of the biological endpoints measured; therefore, the author deemed it unnecessary to perform residue analysis.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP, and Quality Assurance Statements were provided. This study was conducted in compliance with the most recent edition of the Principles of Good Laboratory Practice, Chemikaliengesetz, Attachment 1, Germany, and the OECD Principles of Good Laboratory Practice.

Guideline Criteria	Reported Information
	The German requirements are based on the OECD Principles of GLP, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF, and METI) on the basis of intergovernmental agreements. This study was not conducted according to any established guidelines; therefore, it was performed according to the study plan and SOPs of eurofins-GAB.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Observations of guttation and proportion of guttating plants:
No guttation was observed in the evening. Guttation on adjacent vegetation and on neighboring fields was observed on most days when guttation occurred on the maize plot in the control. No guttation was observed on adjacent vegetation around the treatment plot.
The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. In general, guttation occurred at a similar rate over the 4 zones assessed but not at a similar rate between the control and treatment plots.
In general, the occurrence of guttation was more pronounced in the treatment plot compared to the control plot. Dew and guttation did not occur together on all assessment days. Generally, there were more days with occurrences of guttation only as compared to days with both guttation and dew.

Honeybees visiting plants displaying guttation:
During the assessment of guttation in the control plot, no bees were observed on maize plants drinking or collecting water from guttation droplets or dew in the assessment zones. During 4 assessments out of 63 in the control plot, 1bee was found sitting on the ground. In the 2 m^2 observation areas, bees were found sitting on the ground or on plants on 3 out of 32 assessments.

In the treated plot, 14 bees were observed sitting on plants or on the ground or flying over the crop in 9 of 78 assessments. In the 2 m^2 areas, bees were on the ground or on plants for 3 out of 59 assessments (one single bee per area).

No honeybees were observed consuming guttation liquid in the control or treatment plot for the entire duration of the study period.

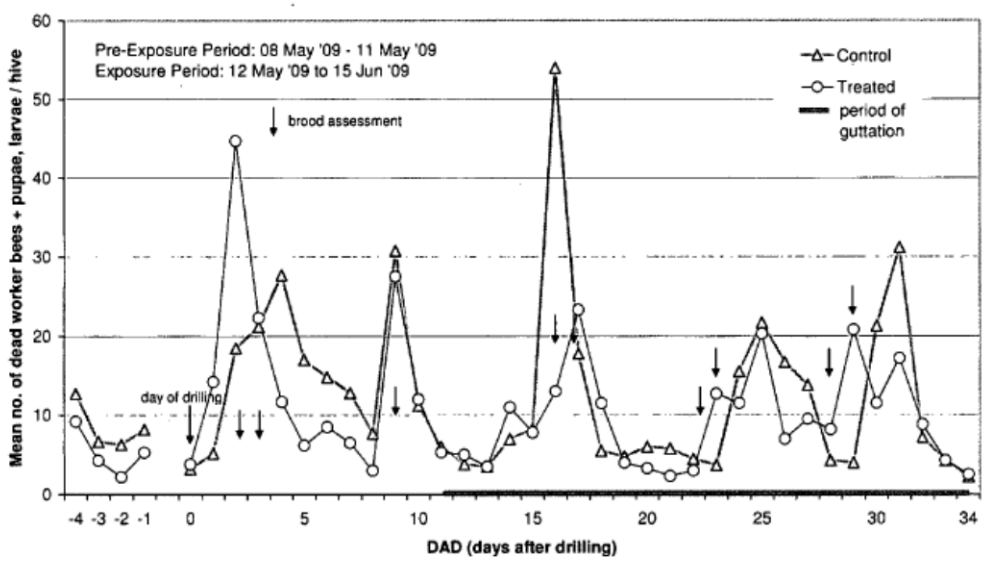
Flight activity:
Flight activity was low in the morning due to low temperatures. Flight activity increased during the course of the morning in both plots. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots. No behavioral anomalies were observed.

Mortality:
The daily mean pre-exposure (days -4 to -1) mortality (linen sheets + dead bee traps) in the control and treatment groups was 8.5 and 5.3 bees/hive, respectively. On the day of drilling (but after the process was complete), mortalities averaged 3.2 dead bees/hive in the control field as compared to 3.8 dead bees/hive in the treated field. One day after drilling, control mortality averaged 5.2 dead bees/hive and treatment mortality averaged 14.2 dead bees/hive.

An increase in mortality was observed on the second day after drilling (DAD+2) in 5 colonies of the treated plot and one colony of the control plot; however, the study author attributed this to the brood assessment that was carried out that morning. For the remaining assessment days, mean daily mortality of both the control and treatment groups fluctuated (Figure 1). Mortality peaks usually occurred simultaneously in both the control and treated plots. Increases in the number of dead bees in front of the hives were mainly observed after the brood assessments that were performed during exposure in both treatment groups. The mean daily mortality during guttation (days 11 to 34 after drilling) was 11.4 and 9.5 dead bees/hive in the control and treated groups, respectively.

The mean daily mortality (linen sheets + bee traps) for the entire exposure (34 days) was 12.7 and 11.1 bees/hive in the control and treated groups, respectively.

Figure 1. *Mean number of dead worker bees, pupae, and larvae/hive/day collected in the dead bee traps and on the linen sheet in front of the hives in the control and treatment groups before drilling and during the time of exposure at the test site.*



Colony condition and brood development:

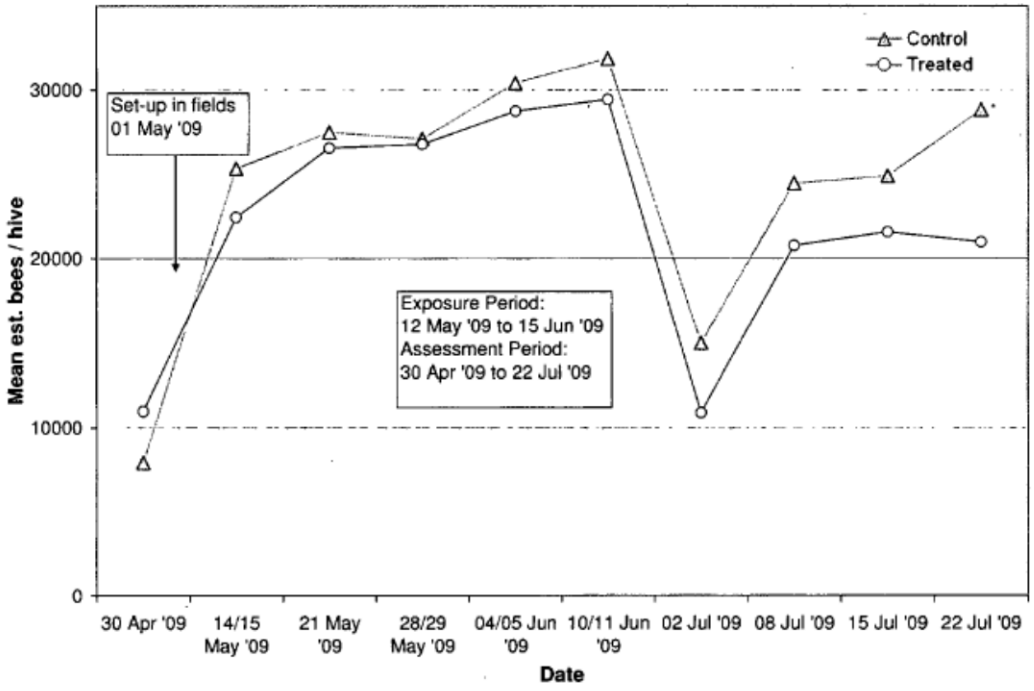
At the first brood assessment, colony strength (=mean number of bees/hive) in the control hives ranged from 5,382 to 11,818 bees. Colony strength in the treatment hives ranged from 5,630 to 15,197 bees. Only the bees that were present in the hives at the time of the assessment were included in the estimates. A portion of the worker bees was outside foraging, so the estimates underestimate actual colony strength.

Colony strength in both the control and treatment group fluctuated over the observation period from the end of April until mid-July; both groups increased or decreased in tandem with one another (Figure 2). In the spring (end of April to May), both colonies increased in size and strength due to an increase in food supply in the near surroundings of the test fields. From mid-May until the end of the exposure period, colony strength was comparable or increased slightly in both the control and treatment groups. After the hives were transported to the monitoring location, a decrease in colony strength was noted in both groups. During the assessments at the monitoring site, most colonies of the treatment group had slightly lower colony strength in comparison to the control. Colony T1 of the treatment group was lower in strength compared to the other colonies starting at the first assessment in April. In contrast, colony T2 only had a lower number of bees at the last assessments. Colonies T4 and T6 only had lower strengths during the post-exposure period at the monitoring site. These trends were explained by observations of swarming that occurred in these colonies between mid-June and early July. There was no swarming observed in the field, but hatched queen cells were found in the hives.

During swarming, colonies lose worker bees and the old queen. A lack of brood was also observed in July in those colonies. However, the colonies were able to produce a new fertile queen, and brood was found again at the assessment at the end of July. One colony in the control group was also without eggs at the first assessment at the monitoring site, but the colony was not able to recover and died at the end of July. Apart from these findings, colony strength in the control and treatment group fluctuated within the range of natural variability and was in the same order of magnitude. The study author reported that there was no evidence of a treatment-related effect.

The development of the mean abundance of brood on the combs (eggs, larvae, and pupae) in the control and treatment group was similar and showed a similar tendency to fluctuate during the observation period (Figures 3 and 4). During the spring, breeding activity increased, and due to good food supply from flowering plants the amount of food stores also increased. Increases in brood in the control group were slightly higher than the treatment group. However, brood area in both the control and treatment groups was at the same level of magnitude. During the exposure period from mid-May to mid-June, the amount of brood on the combs was stable and only slight changes occurred in one treatment group. After colony set-up at the monitoring site, the amount of brood on combs was reduced in both the control and treatment group. The mean amount of brood in the treatment group was slightly lower than in the control group. The study author reported that there were indications that swarming occurred in colonies T4 and T6 of the treatment group, which serves as a possible explanation for the observation. The amount of food available on the combs was similar between the control and treatment group during the entire observation period.

Figure 2. *Mean number of honeybees per hive (=colony strength) in the control and treatment group.*



*mean of 5 hives (colony C4 dead)

Figure 3. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the treatment group.*

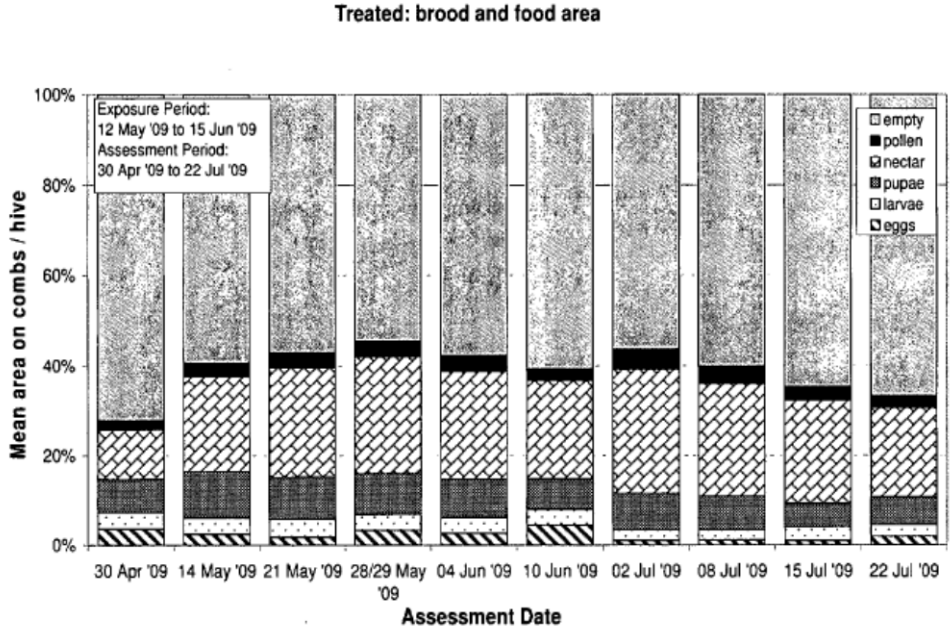
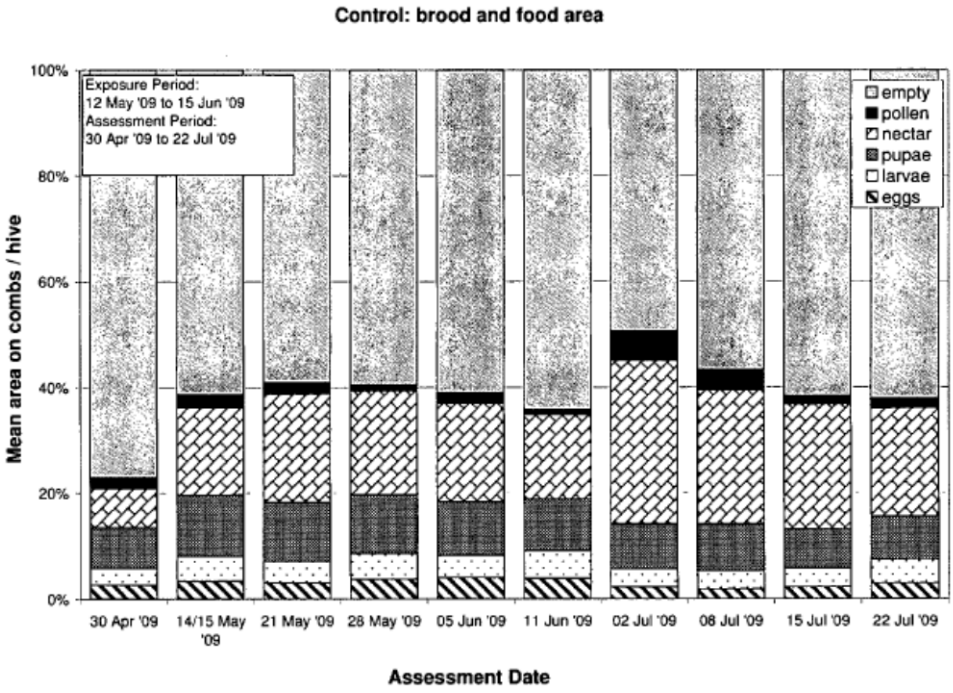


Figure 4. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the control group.*


Control: brood and food area
Exposure Period:
12 May '09 to 15 Jun '09
Assessment Period:
30 Apr '09 to 22 Jul '09
empty
pollen
nectar
pupae
larvae
eggs
100%
80%
60%
40%
20%
0%
Mean area on combs / hive
30 Apr '09
14/15 May '09
21 May '09
28 May '09
05 Jun '09
11 Jun '09
02 Jul '09
08 Jul '09
15 Jul '09
22 Jul '09
Assessment Date

Reported Statistical Results:

The study author did not perform statistical analysis on any of the parameters measured.

14. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Replicate data were provided for the bee trap mortality data when considering each individual hive as a replicate. However, individual hive data was not provided for the mortality data obtained from linen sheets placed in front of each hive. Pre-exposure mortalities were very similar between the control and treatment groups. For the exposure data, there were 8 assessment days where the treatment mortality was possibly biologically significantly higher than in the control group, and in 7 out of 8 assessments mortality was greater than or equal to twice the mortalities observed in the control group. However, levels of mortality were very low in both the control and treatment group; there was likely no effect of the test material.

Overall, the mean mortalities during guttation and the mean mortalities for the entire duration of exposure were very similar between the control and treatment groups; the treatment groups had slightly lower mean mortalities.

The reviewer visually verified the reported results and agrees with the study author's assessments with regard to colony strength. The colony strength in the treatment group was comparable to that of the control group during pre-exposure and for most exposure assessments. The days where colony strength was possibly significantly reduced as compared to the control there was swarming that likely resulted in loss of the queen and numerous worker bees. On the last assessment day, the assessment was so near to the reported dates of swarming that the reviewer doubts that the colony had sufficient time to recover. Therefore, any reductions cannot be conclusively attributed to the drilling of the treated maize seeds.

The reviewer visually assessed the brood and food area data and determined that there were likely no biologically significant differences present either during pre-exposure or during exposure. The cases where there might have been significant reductions in the treatment as compared to the control coincided with the swarming incident that was reported, and thus there was likely an effect on the brood and food area as well.

16. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's. Small differences between treatment and control hive data were found on various dates, but essentially no biologically significant differences in colony strength, mortality, and brood and food area occurred throughout the study.

Climatic data (temperature, humidity, rainfall, and cloud formation) were recorded at the control field plot. Temperature and humidity were recorded at 15 minute intervals using a data logger starting May 27, 2009. Daily rainfall was measured using a rain gauge. Data from May 1 to May 26, 2009 were taken from an official weather station in Mourmelon le Grand. While colonies were located at the monitoring location, weather data were collected from the nearby official government weather station in Hegeney.

Soil samples were collected from the test fields for determination of physico-chemical properties. Five soil cores (5 cm width) were collected to a depth of 20 cm from each corner of the treated and control field plot (4 x 5 samples per field). Standard soil parameters were determined:

	Control	Treatment
Soil Type[6)]	High clay silt	High clay silt
pH value ($CaCl_2$) [1)]	7.4	7.4
WHC_{max} [g /100 g soil dry weight] [2)]	51.7	52.6
CEC [mval Ba/ 100 g soil dry weight] [4)]	21.8	22.4
TOC [%] [3)]	2.08	2.76
Clay [%] (< 0.002 mm) [5)]	21.6	20.8
Silt [%] (0.063 mm to ≥ 0.002 mm) [5)]	69.4	66.9
Sand [%] (2 mm to ≥ 0.063 mm) [5)]	9.0	12.2

WHC_{max} = Maximum Water Holding Capacity
CEC = Cation Exchange Capacity
TOC = Total Organic Carbon
[1)]DIN ISO 10390 mod
[2)]Schaller 1993
[3)]DIN ISO 10694
[4)]Mehlich method mod.
[5)]DIN 19683
[6)]DIN 4220

16. REFERENCES:

DIN 19683 BLATT 3 (1973-04): Physikalische Laboruntersuchungen – Bestimmung der Korngrossenzusammensetzung.

DIN 4220 (2008-11) Bodenkundliche Standortbeurteilung – Kennzeichnung, Klassifizierung und Ableitung von Bodenkennwerten.

DIN ISO 10390 (2005-12): Bodenbeschaffenheit – Bestimmung des pH-Wertes.

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